## In the Specification

Please replace the paragraph beginning on page 19, line 14 with the following amended paragraph:

Based on these data and excluding the six cysteines, we judge that the KuDom structure will allow those substitutions shown in Table 9. The class indicates whether the substitutions: A) are <u>very</u> likely to give a stable protein having substantially the same binding to hNE, hCG, or some other serine protease as the parental sequence, B) are likely to give similar binding as the parent, or C) are likely to give a ~~proteins~~<u>protein</u> retaining the KuDom structure, but which are likely to affect the binding. Mutants in class C must be tested for affinity, which is relatively easy using a display-phage system, such as the one set forth in W0/02809. The affinity of hNE and hCG inhibitors is most sensitive to substitutions at positions 15, 16, 17, 18, 34, 39, 19, 13, 11, 20, 36 of BPTI, if the inhibitor is a mutant of ITI-D1, these positions must be converted to their ITI-D1 equivalents by aligning the cysteines in BPTI and ITI-D1.

Please replace the paragraph beginning on page 35, line 2 with the following amended paragraph:

**Example 9**: Amino-acid sequences of EPI-HNE-3 and EPI-HNE-4

Table 10 gives amino acid sequences of four human-neutrophil-elastase (hNE) inhibitor proteins: EPI-HNE-1 (<u>which is</u> identical to EpiNE1), EPI-HNE-2, EPI-HNE-3, and EPI-HNE-4. These proteins have been derived from the parental Kunitz-type domains shown. Each of the proteins is shown aligned to the parental domain using the six cysteine residues (shaded) characteristic of the Kunitz-type domain. Residues within the inhibitor proteins that differ from those in the parental protein are in upper case. Entire proteins having the sequences EPI-HNE-1, EPI-HNE-2, EPI-HNE-3, and EPI-HNE-4 (Table 10) have been produced. Larger proteins that comprise one of the hNE-inhibiting sequences are expected to have potent hNE-inhibitory activity; EPI-HNE-1, EPI-HNE-2, EPI-HNE-3, and EPI-HNE-4 are particularly preferred. It is expected that proteins that comprise a significant part of one of the hNE-inhibiting sequences found in Table 10 (particularly if the sequence starting at or before the first cysteine and continuing through or beyond the last cysteine is retained) will exhibit potent hNE-inhibitory activity.

Please replace the paragraph beginning on page 36, line 11 with the following amended paragraph:

We have used this high density *Pichia pastoris* production system to produce proteins by secretion into the cell ~~CM~~culture medium. Expression plasmids were constructed by ligating synthetic DNA sequences encoding the *S. cerevisiae* mating factor α prepro peptide fused directly to the amino terminus of the desired hNE inhibitor into the plasmid pHIL-D2 using the *Bst*BI and the *Eco*RI sites shown. Table 24 gives the DNA sequence of a *Bst*BI-to-*Eco*RI insert that converts pHIL-D2 into pHIL-D2(MFα-PrePro::EPI-HNE-3). In this construction, the fusion protein is placed under control of the upstream inducible *P. pastoris aox1* gene promoter and the downstream *aox1* gene transcription termination and polyadenylation sequences. Expression plasmids were linearized by *Sac*I digestion and the linear DNA was incorporated by homologous recombination into the genome of the *P. pastoris* strain GS115 by spheroplast transformation. Regenerated spheroplasts were selected for growth in the absence of added histidine, replated, and individual isolates were screened for methanol utilization phenotype (*mut*$^+$), secretion levels, and gene dose (estimated via Southern hybridization experiments). High level secretion stains were selected for production of hNE inhibitors: PEY-33 for production of EPI-HNE-2 and PEY-43 for production of EPI-HNE-3. In both of these strains, we estimate that four copies of the expression plasmid are integrated as a tandem array into the *aox1* gene locus.

Please replace Table 21, page 89 with the following amended Table:

TABLE 21

**ITI-D1-derived hNE Inhibitors**

WEAK ($K_D > 10^{-8}$ M)

```
                1    1    2    2    3    3    4    4    5    5
         1....5....0....5.....0...5....0....5....0....5....0....5...
1.   KEDSCQLGYSAGPCMGMTSRYFYNGTSMACETFQYGGCMGNGNNFVTEKDCLQTCRGA
```

MODERATE ($10^{-8} > RD\ 22\ 10^{-9}$)
```
2.   KEDSCQLGYSAGPCVAMFPRYFYNGTSMACETFQYGGCMGNGNNFVTEKDCLQTCRGA
3.   RPDFCQLGYSAGPCMGMTSRYFYNGTSMACETFQYGGCMGNGNNFVTEKDCLQTCRGA
```

STRONG ($10^{-9} > KD > 10^{-11}$ D)
```
4.   RPDFCQLGYSAGPCVAMFPRYFYNGTSMACETFQYGGCMGNGNNFVTEKDCLQTCRGA
5.   RPDFCQLGYSTGPCVAMFPRYFYNGTSMACETFQYGGCMGNGNNFVTEKDCLQTCRGA
6.   KEDFCQLGYSAGPCVAMFPRYFYNGTSMACETFQYGGCMGNGNNFVTEKDCLQTCRGA
7.   KPDSCQLGYSAGPCVAMFPRYFYNGTSMACETFQYGGCMGNGNNFVTEKDCLQTCRGA
8.   RPDFCQLGYSAGPCIGMFSRYFYNGTSMACETFQYGGCMGNGNNFVTEKDCLQTCRGA
```

VERY STRONG ($K_D < 10^{-11}$ M)
```
9.   RPDFCQLGYSAGPCVAMFPRYFYNGTSMACQTFVYGGCMGNGNNFVTEKDCLQTCRGA
10.  RPDFCQLGYSAGPCVAMFPRYFYNGASMACQTFVYGGCMGNGNNFVTEKDCLQTCRGA
11.  RPDFCQLGYSAGPCVAMFPRYFYNGTSMACETFVYGGCMGNGNNFVTEKDCLQTCRGA
12.  RPDFCQLGYSAGPCVGMFSRYFYNGTSMACQTFVYGGCMGNGNNFVTEKDCLQTCRGA
```

Residues shown underlined and bold are changed from those present in ITID1

Sequences Key:
```
 1. ITI-D1        SEQ ID NO. 008 16
 2. ITI-D1E7      SEQ ID NO. 009 21
 3. BITI          SEQ ID NO. 030 141
 4. BITI-E7       SEQ ID NO. 010 142
 5. BITI-E7-1222  SEQ ID NO. 012 143
 6. AMINO1        SEQ ID NO. 015 22
 7. AMINO2        SEQ ID NO. 016 23
 8. MUTP1         SEQ ID NO. 014 24
 9. BITI-E7-141   SEQ ID NO. 011 17
10. MUTT26A       SEQ ID NO. 018
11  MUTQE         SEQ ID NO. 017
12  MUT1619       SEQ ID NO. 013 20
```

Please replace Table 65, beginning on page 72 to page 73, with the following amended

Table:

Table 65~~9~~: Definition of Class A, B and C mutations in PCT/US92/01501.

| Classes: | A | No major effect expected if molecular charge stays in range -1 to +1. |
| | B | Major effects not expected, but are more likely than in "A". |
| | C | Residue in the binding interface; any change must be tested. |
| | X | No substitution allowed. |

| Res. Id. | EpiNE1 (SEQ ID NO:7) | Substitutions | Class |
|---|---|---|---|
| 1 | R | any | A |
| 2 | P | any | A |
| 3 | D | any | A |
| 4 | F | Y, W, L | B |
| 5 | C | C | X |
| 6 | L | non-proline | A |
| 7 | E | L, S, T, D, N, K, R | A |
| 8 | P | any | A |
| 9 | P | any | A |
| 10 | Y | non-proline prefr'd | B |
| 11 | T | any | C |
| 12 | G | must be G | X |
| 13 | P | any | C |
| 14 | C | C strongly preferred, any non-proline | C |
| 15 | I | V, A | C |
| 16 | A | | C |
| 17 | F | L, I, M, Y, W, H, V | C |
| 18 | F | Y, W, H | C |
| 19 | P | any | C |
| 20 | R | non-proline prefr'd | C |
| 21 | Y | F & Y most prefr'd; W, I, L prefr'd; M, V allowed | C |
| 22 | F | Y & F most prefr'd; non-proline prefr'd | Y, F B |
| 23 | Y | Y & F strongly prefr'd | F ,Y B |
| 24 | N | non-proline prefr'd | A |
| 25 | A | any | A |

| 26 | K | any | A |
|----|---|-----|---|
| 27 | A | any | A |
| 28 | G | non-proline prefr'd | A |
| 29 | L | non-proline prefr'd | A |
| 30 | C | must be C | X |
| 31 | Q | non-proline prefr'd | B |
| 32 | T | non-proline prefr'd | B |
| 33 | F | F very strongly prefr'd; Y possible | X |
| 34 | V | any | C |
| 35 | Y | Y most prefr'd; W prefr'd; F allowed | B |

Res.

| Id. | EpiNE1 | Substitutions | Class |
|-----|--------|---------------|-------|
| 36 | G | G strongly prefr'd; S, A prefr'd; | C |
| 37 | G | must be G so long as 38 is C | X |
| 38 | C | C strongly prefr'd | X |
| 39 | M | any | C |
| 40 | G | A,S,N,D,T,P | C |
| 41 | N | K,Q,S,D,R,T,A,E | C |
| 42 | G | any | C |
| 43 | N | must be N | X |
| 44 | N | S,K,R,T,Q,D,E | B |
| 45 | F | Y | B |
| 46 | K | any non-proline | B |
| 47 | S | T, N, A, G | B |
| 48 | A | any | B |
| 49 | E | any | A |
| 50 | D | any | A |
| 51 | C | must be C | X |
| 52 | M | any | A |
| 53 | R | any | A |
| 54 | T | any | A |
| 55 | C | must be C | X |
| 56 | G | any | A |
| 57 | G | any | A |
| 58 | A | any | A |

prefr'd stands for preferred.

Please replace Table 11, beginning on page 80 to page 81, with the following amended Table:

```
Table 11: Restriction sites in plasmid pHIL-D2


pHIL-D2, 93-01-02       Ngene =  8157


Non-cutters


AflII    ApaI     AscI     AvaI     AvrII    BamHI    BglII

Bsp120I  BsrGI    BssHII   BstEII   FseI     MluI     NruI

PacI     PmlI     RsrII    SacII    SexAI    SfiI     SgfI

SnaBI    SpeI     Sse8387I XhoI(PaeR7I)      XmaI(SmaI)


Cutters


AatII GACGTc                     1    5498

AflIII Acrygt                    1    7746

AgeI Accggt                      1    1009

BlpI GCtnagc                     1     597

BspEI(BspMII,AccIII) Tccgga      1    3551

BspMI gcaggt                     1    4140

Bst1107I GTAtac                  1    7975

BstBI(AsuII) TTcgaa              2     945 4780

Bsu36I CCtnagg                   1    1796

Ecl136I GAGctc                   1     216

EcoRI Gaattc                     1     956

EspI(Bpu1102I) GCtnagc           1     597

HpaI GTTaac                      1    1845

NcoI Ccatgg                      1    3339

NdeI CAtatg                      1    7924

NsiI(Ppu10I) ATGCAt              1     684

PflMI CCANNNNntgg                1     196 (SEQ ID NO:127)
```

```
PmeI    GTTTaaac                        1     420
PstI    CTGCAg                          1    6175
PvuI    CGATcg                          1    6049
SapI    gaagagc                         1    7863
SacI    GAGCTc                          1     216
SalI    Gtcgac                          1    2885
ScaI    AGTact                          1    5938
SphI    GCATGc                          1    4436
StuI    AGGcct                          1    2968
SwaI    ATTTaaat                        1    6532
Tth111I GACNnngtc                       1    7999
XbaI    Tctaga                          1    1741
XcmI    CCANNNNNnnnntgg                  1     711  (SEQ ID NO:128)
```

```
Aox1 5'  1 to about 950
Aox1 3'  950 to about 1250
His4     1700 to about 4200
Aox1 3'  4500 to 5400
bla      5600 to 6400
f1 ori   6500 to 6900
```

Please replace Table 630 on page 123 with the following amended Table:

**Table ~~630~~40**: Temperature stability of EPI-HNE proteins

| Temperature (°C) | Residual hNE Inhibitory Activity | | | |
|---|---|---|---|---|
| | EPI-HNE-1 | EPI-HNE-2 | EPI-HNE-3 | EPI-HNE-4 |
| 0 | 97 | 101 | 96 | 100 |
| 23 | 100 | 103 | 105 | 103 |
| 37 | 100 | 97 | 99 | 98 |
| 45 | 103 | | | |
| 52 | | 101 | 100 | |
| 55 | 99 | | | 98 |
| 65 | 94 | 95 | 87 | |
| 69 | | | | 82 |
| 75 | 100 | | | |
| 80 | | 101 | 79 | |
| 85 | 106 | | | 63 |
| 93 | | 88 | 57 | |
| 95 | 64 | | | 48 |

Proteins were incubated at the stated temperature for 18 hours in buffer at pH 7.0. In all cases protein concentrations were 1 µM. At the end of the incubation period, aliquots of the reactions were diluted and residual hNE-inhibition activity determined.